Enzymatic mineralization of hydrogels with calcium and magnesium phosphate

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Introduction The enzyme alkaline phosphatase (ALP) induces hydrogels' mineralization with calcium phosphate (CaP), but mineralization with magnesium phosphate (MgP) remains unexplored. In this study, enzymatic mineralization of gellan gum (GG) hydrogels was induced by incubation in solutions of calcium and magnesium glycerophosphate (CaGP, MgGP). ***Materials and Methods*** GG hydrogels containing ALP were mineralized for 7 d at different CaGP:MgGP concentration ratios, namely 0.1:0 (A), 0.075:0.025 (B), 0.05:0.05 (C), 0.025:0.075 (D) and 0:0.1 (E) M. After mineralization, gels were rinsed and incubated for 1 d in Milli-Q water to remove residual CaGP and MgGP. The dry mass percentage, which served as a measure of the extent of mineral formation, was calculated as: (weight after incubation and subsequent freeze-drying/weight after incubation but before freeze-drying)*100. Samples were characterized by TGA, FTIR, Raman, XRD, SEM, EDS, ICP-OES and compressive testing. Cytocompatibility was evaluated by culturing HFF-1 cells in eluate from samples and measuring viability by MTT Assay. Adhesion and proliferation were assessed by MTT Assay 1 and 11 d post-seeding using MC3T3-E1 cells (10^5 cells/sample). Cell attachment was evaluated by live/dead staining. ***Results and Discussion*** EDS, ICP-OES, dry mass percentage and TGA measurements showed that more Ca than Mg was incorporated into mineral. Also, morphology visualized by SEM and FTIR, Raman and XRD spectra (Figure a) of mineral formed in samples incubated in mineralization solutions containing CaGP (A, B, C, D) resembled CaP more strongly than MgP. Young's modulus was dependent on the medium composition (Figure b). (A) and (B) groups were significantly stiffer than the (C) and (D) groups, while the (E) group was at least three times stiffer than all other groups. Mineralization led to far superior adhesion of viable cells. After 1 d, viability was highest on the (E) group and lowest for the (A) group. After 11 d, viability was similar on all samples except for the (A) group which displayed markedly lower viability (Figure c). Higher proliferation on samples containing Mg might be caused by a stimulatory effect of Mg. ***Conclusion***. Formation of CaP was favourised more strongly than MgP. Mineralization led to an increase in stiffness, which was greatest for the (E) group. MgP enhanced attachment and proliferation.

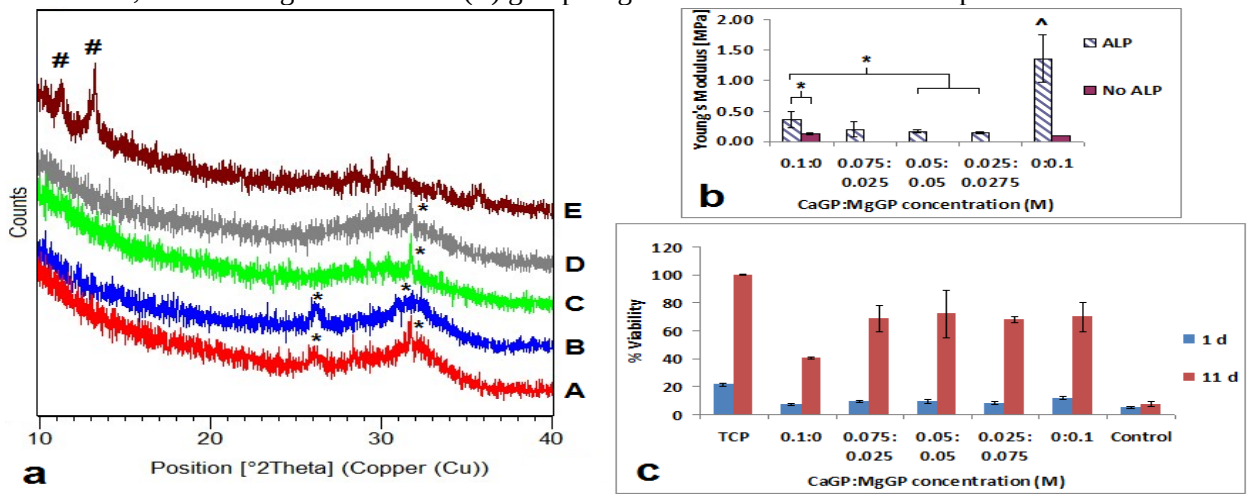

Figure. Analysis of gels mineralized at CaGP:MgGP concentrations: 0.1:0 (A), 0.075:0.025 (B), 0.05:0.05 (C), 0.025:0.075 (D), 0:0.1 (E) M. (a) XRD analysis. Peaks for apatite (*) and bobierrite (#)are marked. (b) Young's Modulus. ^: $p < 0.001$ relative to all other groups; *: $p < 0.01$. (c) Viability of MC3T3-E1 at 1, 11 d. Control: ALP-free GG. TCP: Tissue culture polystyrene.